

## Variability analysis of the Phenotypic Characteristics of some Grapefruit and Pomelo under Moroccan Conditions

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Citrus is an economically important fruit crop with a long history of cultivation worldwide, and many citrus varieties are grown extensively in Morocco, both for domestic consumption and export. Nevertheless, the genetic diversity of this genus in Morocco has not been reported upon. Our objective was to explore phenotypic variations in grapefruit and pomelo genotypes for targeted breeding, enhancing key traits, and identifying opportunities for genetic improvement. We emphasize practical implications and outline future research directions for developing improved citrus varieties. This present study physicochemically analyzes eight grapefruit (*Citrus paradisi*) and two pomelo (*Citrus maxima*) cultivars to assess the genetic variability of these fruits' physicochemical characteristics. The results revealed significant variance among the genotypes for all traits. For example, the phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the traits. Moreover, a high degree of heritability was recorded for seed number,  $\beta$ -carotene, vitamin C, juice yield (%), total soluble solids, titratable acidity, maturity index, fruit diameter, and fruit weight, whereas limited heritability was observed for fruit length, peel thickness, and segment number per fruit. The high genetic advance scores corresponded to the high degree of heritability estimates for seed number per fruit,  $\beta$ -carotene, juice yield, and vitamin C, suggesting the existence of additive genetic effects. Our principal component analysis (PCA) identified four components with cumulative variance of 93.20%, while hierarchical cluster analysis grouped the collection into five clusters. In conclusion, phenotypic distinctions among grapefruit and pomelo genotypes reveal opportunities for genetic enhancement through selection or hybridization. Varied traits, including seed count, vitamin C, beta-carotene, and juice yield, across genotypes suggest potential avenues for targeted breeding and practical applications in diverse citrus varieties.

Keywords: Citrus maxima, citrus paradisi, GCV, heritability, PCV, vitamin C.

### INTRODUCTION

Citrus is a very important fruit crop in the world. It belongs to the *Rutaceae* family and covers a large botanical family that has derived through natural or induced mutations, as well as crossing and selecting from the original species and their progenies. They include, for example, *Citrus sinensis* (sweet orange), *Citrus aurantium* (bitter orange), *Citrus reticulata* (mandarin), *Citrus limon* (lemon), *Citrus aurantifolia* (lime), *Citrus grandis* (pomelo), *Citrus bergamia* (bergamot), and *Citrus paradisi* (grapefruit) (Tadeo *et al.*, 2008; Inglese and Sortino, 2019). These fruits are widely recognized for their organoleptic, nutritional and health properties, both as fresh

fruits and juices (Lado *et al.*, 2018). Among the main citrus varieties is the grapefruit (*Citrus Paradisi Macfadyen*), which is a natural hybrid of the pummelos (*C. grandis* L.BSF) and the sweet orange (*C. sinensis* L.OSB). It originated on the Caribbean Island of Barbados in the West Indies around 1750 (Hodgson, 1967; Scora *et al.*, 1982), while the pomelo (*C. grandis* L.BSF) originated in Southeast Asia (Polynesia and Malaysia) (Wu *et al.*, 2018), where it is still widely grown and consumed. The main areas of production in the east are southern China, southern Japan, Thailand, Vietnam, Malaysia, and Indonesia. Pomeles are also grown in the USA (California and Florida), the Caribbean islands, and Africa (Hodgson, 1967).

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pomelo and grapefruit species contain antioxidant compounds that are believed to provide many human health benefits, including for preventing cancer and cardiovascular disease. This is partly reflected by the vitamin C and carotenoids content (Rajendran *et al.*, 2014), with carotenoids being one of the most important phytochemicals in citrus. In addition, the composition of a fruit's carotenoids is a determining factor in their commercial acceptance (Tadeo *et al.*, 2020). Indeed, citrus is considered one of the most complex sources of carotenoids, with there being a large diversity of carotenoids among the various species and cultivars (Rodrigo *et al.*, 2013). In addition, carotenoid content and profiles are influenced by genotypes and, in general, mandarin and orange cultivars tend to accumulate carotenoid concentrations more so than grapefruits, pomelos, and lemons (Rodrigo *et al.*, 2013).

The genetic diversity of citrus fruits results from a variety of causes, including mutation, hybridization, and the mode of reproduction, which is mainly apomictic. Many researchers (Barkley *et al.*, 2006; Garcia-Lor *et al.*, 2013) have reported genetic diversity in citrus fruits, such as for lemons (Gulsen and Roose, 2001), sweet oranges (Novelli *et al.*, 2006), grapefruits (Corazza-Nunes *et al.*, 2002), and pummelos (Barkley *et al.*, 2006). The last study revealed that pomelo has a greater degree of genetic diversity than other species, and morphological and genetic diversity are important for cultivar characterization and screening. Although spontaneous variation in bud sport has been widely employed in grapefruit, with numerous novel cultivars having been commercialized, little is known about their behavior, fruit quality, and production under local agroclimatic circumstances. The majority of commercial cultivars originate from natural plant breeding, such as through selection, natural bud sports, interploidy hybridization, and mutations (Anderson and Krathwohl, 2000; Usman and Fatima, 2018).

A dozen cultivars generated from pomelos—such as grapefruits, tangelos, and hybrids—have been the subject of publications in recent years, with the result being a crucial collection of genes for the growing of new varieties of citrus (Nicolosi *et al.*, 2000; Barkley *et al.*, 2006; Garcia-Lor *et al.*, 2013). In this context, the National Institute for Agricultural Research (INRA) in Kenitra, Morocco has created a number of collections of citrus-related genetic resources that play an important role in maintaining diversity while facilitating access to them for constantly developing new varieties. This study explores phenotypic variations in grapefruit and pomelo genotypes to guide genetic improvement through targeted breeding. Identify opportunities to enhance key traits. Establish correlations for genotype selection in breeding programs. Emphasize practical implications and outline future research directions, focusing on applying genetic insights to develop diverse and improved citrus varieties.

## MATERIALS AND METHODS

**Experimental Site:** The experiment was conducted at the National Institute for Agricultural Research's (INRA) experimental field in Kenitra, Morocco. The site is geographically located at an altitude of approximately 25 m and a latitude of 34°64. The climate is Mediterranean, sub-humid, and moderate in nature, and it experiences a mild winter with few frosts. The soil there is sandy (98 %) on the surface and sandy-clay at depth.

**Plant Materials:** Ten different genotypes of grapefruit and pomelo were used as experimental materials. Among these genotypes, eight different grapefruit genotypes—namely Jouva, Marsh, Foster, McCarty, Natsu Mikan, Ruby, Thompson, and Triumphe (*Citrus paradisi* Macf), as well as two pomelo genotypes, Pubescent and Sidi Aissa (*Citrus maxima* (Burm.) Merr.)—were grafted onto sour orange (*Citrus aurantium* L.) rootstocks (Table 1). The fruits were collected during the 2019–2020 season from adult trees of a uniform age of 15 years that had been subjected to the same agricultural practices.

**Table 1. List of the ten genotypes included in the study.**

Sr.	Genotype	Species	Parentage	Common name
1	Ruby	<i>C. paradisi</i> Macfad	<i>C. sinensis</i> x <i>C. grandis</i>	Grapefruit
2	Jouva	<i>C. paradisi</i> Macfad	<i>C. sinensis</i> x <i>C. grandis</i>	Grapefruit
3	Mac carty	<i>C. paradisi</i> Macfad	<i>C. sinensis</i> x <i>C. grandis</i>	Grapefruit
4	Sidi Aissa	<i>C. grandis</i> Osbeck	Female parent	pomelo
5	Pubescent	<i>C. grandis</i> Osbeck	Female parent	pomelo
6	Foster	<i>C. paradisi</i> Macfad	<i>C. sinensis</i> x <i>C. grandis</i>	Grapefruit
7	Thompson	<i>C. paradisi</i> Macfad	<i>C. sinensis</i> x <i>C. grandis</i>	Grapefruit
8	Triumphe	<i>C. paradisi</i> Macfad	<i>C. sinensis</i> x <i>C. grandis</i>	Grapefruit
9	Marsh	<i>C. paradisi</i> Macfad	<i>C. sinensis</i> x <i>C. grandis</i>	Grapefruit
10	Natsu Mikan	<i>C. paradisi</i> Macfad	<i>C. sinensis</i> x <i>C. grandis</i>	Grapefruit

**Physical and Chemical Determinations:** Data for the various physicochemical parameters of the fruits and fruit juice were collected in terms of fruit weight, fruit diameter, fruit length, titratable acidity (TA), total soluble solids (TSS), fruit peel thickness, seed number per fruit, segment number per fruit, and fruit maturity stage (TSS/TA) based on suggested indicators. The physicochemical study of the fruit samples was performed in the National Institute for Agricultural Research's laboratory at the Research Unit for Plant Breeding and Germplasm Conservation (INRA) in Kenitra in



accordance with scientific standards and methodologies. Nine fruits for each genotype were collected randomly from different directions to record different observations, with such observations being recorded for each fruit separately. Fruit weight was measured using an electric scale, while parameters such as fruit diameter, fruit length, and peel thickness were recorded using a digital caliper (Mitutoyo Inc., Japan). The seed and segment numbers per fruit were counted, while the total soluble solids in the juices were calculated using a PR-201a digital refractometer (Atago, Tokyo, Japan). Titratable acidity was calculated through titration with 0.1 N NaOH solution with phenolphthalein as an indicator before being expressed as grams of citric acid per 100 ml juice. The juices were squeezed on the same day, filtered through a 1 mm mesh screen, and stored in amber bottles at -18°C until later analysis. Each measurement was replicated three times, resulting in a total of nine fruits per measurement.

**Determining the Carotenoid Content of Juice:** The total carotenoids were determined using the method outlined by (Lee *et al.*, 2001), such that 2 mL of each kind of fruit juice was combined with five mL of the extraction solvent (hexane, acetone, ethanol, 50: 25: 25, v/v/v). After shaking, the mixture was centrifuged for 5 minutes at 6500 rpm. The top layer of carotenoid-containing hexane was collected and put in a 25 ml flask. The volume of this hexane recovered was then topped up to 25 ml with blank hexane. A spectrophotometer (SP-8001.Metertech Inc. 1.09) was used to measure absorbance at 450 nm, with the carotenoid levels being quantified in milligrams of  $\beta$ -carotene per liter. The  $\beta$ -carotene extinction coefficient ( $E_{1/2} = 2505$ ) was used to calculate the total carotenoids. Each measurement comprised three replications, each of which used juice from three different fruits, making for a total of nine fruits per measurement.

**Determining the Ascorbic Acid (Vitamin C) Content:** The technique described by (Izuagie and Izuagie, 2007) was used to determine the vitamin C concentration. In a 500-mL bottle of distilled water, we dissolved 0.02 g of KIO<sub>3</sub> and 1.06 g of KI. Some 1 mL of concentrated tetraoxosulphate (VI) (H<sub>2</sub>SO<sub>4</sub>) acid was added to the solution, thus acidifying it. After swirling the combination, the solution's volume was increased to 500 ml with purified water. The bottle was then plugged and agitated to ensure that its contents were homogeneous. Consequently, the concentration of iodine in the solution was  $5.6076 \times 10^{-3}$  M, so each sample was titrated with 20 mL of juice against a standard iodine solution of  $5.6076 \times 10^{-3}$  mol. L<sup>-1</sup>. A starch solution was utilized to give an indication. Each measurement comprised three replications, each of which used the juice from three different fruits, making for a total of nine fruits per measurement.

**Statistical Analysis:** Data for the continuous variables were provided as means  $\pm$  standard deviations. A Student's t-test or Welch's t-test was used to examine the mean differences between two sample groups, as defined by the variety group,

when the variables were regularly distributed. For non-normally distributed variables, the Mann Whitney U test was applied. To ascertain the significance of any differences between the tested physicochemical indicators, the non-parametric Kruskal-Wallis test and multiple comparisons of mean ranks were used. This non-parametric test was applied because of the lack of a normal distribution for most of the physicochemical indicators being analyzed, as was shown by the Shapiro-Wilk test, as well as the variance inequality that was revealed by the Fisher-Snedecor test. ANOVA was applied to analyze any changes in physicochemical properties, while significant differences between means were compared by Bonferroni test at a significance level of 0.05. The results were deemed to be statistically significant when the P-value was lower than 0.05 ( $p < 0.05$ ). To determine which variables most correctly characterized the physicochemical variety of the pomelos and grapefruits, the principal components with the highest eigenvalues ( $>1.0$ ) were chosen in the manner suggested by (Jeffers, 1967). Principal component analysis (PCA) was performed using the ggplot2, factoextra, and FactoMineR packages (Lê *et al.*, 2008 ; Wickham and Wickham, 2016). For hierarchically clustering the principal components and evaluating the clustering tendency, we determined the optimal number of clusters, calculated the cluster-validation statistics, choose the best clustering algorithms, and calculated the p-value for hierarchical clustering (Bezdek and Hathaway, 2002; Suzuki and Shimodaira, 2004; Kaufman and Rousseeuw, 2009) using the pvclust package and HCPC () function. Variability was assessed using genotypic variances and coefficients of variation, as proposed by (Burton and Devane, 1953), while the estimated of phenotypic coefficient of variation (PCV) and genetic coefficient of variation (GCV) were calculated using the method reported by (Singh and Chaudhary, 1985). The broad-sense heritability ( $H^2$ ) of all characteristics was calculated using the method of (Allard, 1960) and genetic advance as a percentage of population means (GAM) was calculated using the method outlined by (Johnson *et al.*, 1955). According to (Singh *et al.*, 2001), heritability can be divided into four categories, namely low (40%), medium (40–59%), fairly high (60–79%), and extremely high (80%). We used the library function "library" to load the variability package (variability in (R: The R Project for Statistical Computing (r-project.org), which in turn activated all the functions residing in the package. This variability package has a function named gen.var () that helped us to identify genetic parameters. The degree of association between the studied characteristics was determined according to the correlation coefficients between them. The correlation coefficient matrix and the "correlation heatmap" were visualized using the "ggpair" function of the GGally and ggplot2 packages (Emerson *et al.*, 2013). Three levels of significance (0.05, 0.01, and 0.001) were employed in calculating correlation coefficients.



All statistical analyses were performed using the R software (<https://www.r-project.org/>) with the “pheatmap” package (Kolde and Kolde, 2015), while SPSS V26 was used to analyze variance. All of the reported p-values were two-tailed with a statistical significance of  $p < 0.05$ .

## RESULTS

**Table 2. Genotypic diversity for the quantitative physicochemical characteristics of the ten different grapefruit and pomelo cultivars.**

Varieties/ Pairwise comparisons**	Segments Number	fruit weight (g)	Fruit diameter (mm)	Fruit length (mm)	Peel thickness (mm)	Seeds Number
Foster	(1)59.90 <sup>ab</sup>	497.7±78.8 <sup>abc</sup>	105.85±8.19 <sup>abc</sup>	92.45±5.55 <sup>a</sup>	9.18±1.4 <sup>bc</sup>	13.33±0.58 <sup>ab</sup>
Jouva	(1)45.45 <sup>ab</sup>	684.8±113.8 <sup>a</sup>	112.93±8.12 <sup>abc</sup>	100.04±9.96 <sup>ab</sup>	9.04b±1.8 <sup>c</sup>	13.33±1.53 <sup>ab</sup>
MacCarty	(19)62.25 <sup>ab</sup>	604.4±49.9 <sup>ab</sup>	108.96±3.07 <sup>abc</sup>	90.49±8.75 <sup>ab</sup>	8.04±1.2 <sup>c</sup>	14.00±0.00 <sup>a</sup>
Marsh	(0)60.45 <sup>ab</sup>	399.4±57.7 <sup>bc</sup>	97.87±4.49 <sup>c</sup>	80.46±11.73 <sup>abc</sup>	8.3±1.6 <sup>c</sup>	14.00±1.00 <sup>a</sup>
Natsu Mikan	(1)64.25 <sup>ab</sup>	362.6±73.5 <sup>c</sup>	95.11±4.77 <sup>c</sup>	81.9±12.53 <sup>abc</sup>	9.2±1.560 <sup>bc</sup>	13.33±0.58 <sup>ab</sup>
Pubescent	(73)28.35 <sup>a</sup>	572.1±100.2 <sup>abc</sup>	117.79±7.11 <sup>ab</sup>	109.04±10.3 <sup>abc</sup>	14±0.9 <sup>a</sup>	12.67±0.58 <sup>ab</sup>
Ruby	(0)48.40 <sup>ab</sup>	679.1±23.7 <sup>a</sup>	112.21±4.87 <sup>abc</sup>	107.86±8.05 <sup>abc</sup>	11±0.9 <sup>b</sup>	12.67±0.58 <sup>ab</sup>
Sidi Aissa	(66)69.85 <sup>b</sup>	624.3±66.6 <sup>ab</sup>	122.793±0.8 <sup>a</sup>	114.2±5.91 <sup>abc</sup>	13.5±0.7 <sup>a</sup>	14.00±1.00 <sup>a</sup>
Thompson	(0)23.55 <sup>a</sup>	503.5±52.3 <sup>abc</sup>	102.67±8.04 <sup>bc</sup>	93.4±11.92 <sup>bc</sup>	9.2±1.6 <sup>bc</sup>	12.33±0.58 <sup>b</sup>
Triomphe	(1)42.55 <sup>ab</sup>	439±113.4 <sup>bc</sup>	99.38±8.41 <sup>c</sup>	87.81±5.92 <sup>c</sup>	9.15±2.2 <sup>bc</sup>	12±1 <sup>b</sup>
Max	74.00	799.90	123.77	118.72	14.70	15.00
Min	00.00	311.60	91.90	68.51	5.40	11.00
Overall mean	16.26	536.70	107.55	95.77	10.08	13.16
Kruskal-Wallis test/ one-way Anova	Test value/F	28.04	6.39	6.16	4.58	4.74
	Test (p)	P>0.05 <sup>C</sup>	P<0.001 <sup>B</sup>	P<0.001 <sup>B</sup>	P<0.001 <sup>B</sup>	P<0.01 <sup>A</sup>

**Continued**

Varieties/Pairwise comparisons**	$\beta$ -carotene (mg /L)	Vitamin C (mg /L)	Juice yield (%)	Titratable acidity (%)	Total Soluble Solids (%)	Maturity Index
Foster	(0)7.50 <sup>b</sup>	516.53±5.7 <sup>d</sup>	(36.67)17.00 <sup>bc</sup>	(1.7)26ab	9.03±0.06 <sup>bc</sup>	(5.29)3.00a
Jouva	(0)7.50 <sup>b</sup>	398.09±5.7 <sup>ef</sup>	(39.36)26.00 <sup>bd</sup>	(1.45)16.5 <sup>bcd</sup>	8.7±0.1 <sup>cd</sup>	(6)11.67ab
Mac carty	(0.18)28.83 <sup>b</sup>	395.87±3.8 <sup>ef</sup>	(34.85)8.00 <sup>cb</sup>	(1.85)29a	10.33±0.31a	(5.56)8.00ab
Marsh	(0.101)23.83 <sup>b</sup>	361.9±5.7 <sup>a</sup>	(40.13)29.00 <sup>b</sup>	(1.56)22abc	9.47±0.06b	(6.05)13.67ab
Natsu Mikan	(0.049)17.67 <sup>b</sup>	591.2±9b	(36.93)20.00 <sup>cb</sup>	(1.45)16.5 <sup>bcd</sup>	7.8±0.2e	(5.38)4.00a
Pubescent	(0.038)21.50 <sup>b</sup>	542.85±0.4 <sup>b</sup>	(26.77)5.00 <sup>cd</sup>	(1.15)9cd	9.1±0.2bc	(7.28)29.00b
Ruby	(0.023)20.17 <sup>b</sup>	381.64±5.7 <sup>g</sup>	(36.26)14.00 <sup>abc</sup>	(1.29)11cd	8.33±0.06de	(6.43)20.83ab
Sidi Aissa	(0)10.00 <sup>b</sup>	536.27±5.7 <sup>c</sup>	(19.66)2.00 <sup>ac</sup>	(1.12)9.5cd	8.9±0.2c	(7.01)26.00ab
Thompson	(0)10.50 <sup>b</sup>	460.6±5.7 <sup>e</sup>	(35.54)11.00 <sup>abc</sup>	(1.26)10cd	8.1±0.37e	(6.43)21.17ab
Triomphe	(0)7.50 <sup>b</sup>	391.51±5.7 <sup>f</sup>	(38.57)23.00 <sup>abc</sup>	(1.15)5.5d	7.2±0.1f	(6.26)17.67ab
Max	0.19	600.20	40.22	1.89	10.60	7.28
Min	0.00	355.32	19.16	1.10	7.10	5.20
Overall mean	0.04	457.64	34.47	1.42	8.69	6.18
Kruskal-Wallis test/ one-way Anova	Test value/F	24.01	633.77	28.79	21.95	63.1013
	Test (p)	P<0.01 <sup>A</sup>	P<0.01 <sup>A</sup>	P<0.01 <sup>A</sup>	P<0.001 <sup>B</sup>	P<0.01 <sup>A</sup>

Note: Data are averaged  $\pm$  SD. <sup>A</sup> Statistical value in italics refer to statistically significant differences at  $p < 0.01$ ; <sup>B</sup> Statistical values in italics mean statistically significant differences at  $p < 0.001$ ; <sup>C</sup> Statistical values in italics mean statistically no significant difference at  $p > 0.05$ . Averages that do not share the same letter are significantly different according to Duncan's multiple range test ( $p < 0.05$ ). Average ranks that do not share the same letter are significantly different. \*\*Significance values were adjusted through Bonferroni correction with multiple tests, and standard deviations are not shown in the table. Note that we only mention the mean ranges and means for the Kruskal-Wallis test.



hierarchical clustering and principal component analysis, we endeavored to group together the most similar and top-performing genotypes based on these twelve physicochemical attributes. This experimental study is oriented towards enhancing the performance of our varieties through a thorough examination of their genetic and phenotypic characteristics across this diverse range of quantitative parameters.

The analysis of variance /Kruskal-Wallis test for twelve quantitative physicochemical characteristics of ten grapefruit and pomelo genotypes revealed a statistically significant difference ( $p < 0.05$ ) across the traits, except for the average seed number per fruit ( $p > 0.05$ ). The weight of individual fruits varied significantly depending on the genotype, with them ranging from 311.60 g to 799.90 g. The heaviest fruit (684.82 g) was found for the Jouva genotype, followed by Ruby (679.14 g), Sidi Aissa (624.26 g), McCarty (604.38 g), Pubescent (572.10 g), and Thompson (503.47 g), while the

lightest (362.62 g) was recorded for the Natsu Mikan genotype. The highest fruit diameter (122.79 mm) and fruit length (114.20 mm) were observed for the *Sidi Aissa* genotype (h, while the average fruit diameter (95.11 mm) and fruit length (80.46 mm) were lowest for the *Natsu Mikan* and *Marsh* varieties, respectively. Skin thickness also differed significantly between genotypes. The *Sidi Aissa* genotype had the thickest skin (13.80 mm), while the thinnest skin (8.04 mm) was observed for the *McCarty* genotype (Table 2). In terms of the number of seeds per fruit, the different genotypes differed significantly. The greatest number of seeds per fruit was recorded for the *Pubescent* genotype (73), followed by the *Sidi Aissa* genotype (66), while the lowest number of seeds per fruit was found for the *Marsh*, *Ruby*, and *Thompson* genotypes (0.00) (Table 2). Nevertheless, no significant differences were obtained among the studied genotypes for the average number of segments per fruit (Table 2).

**Table 3. Analysis of variance (ANOVA) for different physicochemical characteristics in the grapefruit and pomelo cultivars.**

Sr.	Characteristic	Genotypic mean sum square (df=9)	Replication mean sum square (df=2)	Error (df=18)	Coefficient of variance (%)
1	Fruit weight (g)	39022.000***	1385.0000	6628.0000	23.8
2	Fruit diameter (mm)	243.924***	40.6770	39.4480	9.4
3	Fruit length (mm)	404.930**	6.3800	97.6000	14.3
4	Peel thickness (mm)	14.679**	1.2212	3.3055	25.7
5	Segment number	1.574ns	0.2333	0.7518	7.5
6	Seed number	2471.100**	4.0300	2.1100	170.4
7	Vitamin C (mg /L)	20478.700***	21.6000	33.5000	17.5
8	$\beta$ -carotene (mg /L)	0.010***	0.0003	0.0008	155.2
9	Juice yield (%)	122.819***	0.0440	0.0490	17.9
10	Titratable acidity (%)	0.150***	0.0251	0.0123	16.6
11	Total soluble solids ( $^{\circ}$ Brix)	2.375***	0.0339	0.0380	10.0
12	Maturity index	1.280***	0.0053	0.0144	10.3

\*\* indicates significance at the 0.05 probability level. \*\*\* indicates high significance at the 0.01 probability level. NS indicates non-significance at the 0.05 probability level.

**Table 4. Variability of fruit quality parameters among the pomelo and grapefruit cultivars.**

Characteristic	GV	PV	GCV (%)	PCV (%)	$H^2(B)$ %	GA	GAM (%)
Fruit weight (g)	10797.90	17426.00	19.36	24.60	61.96	168.50	31.40
Fruit diameter (mm)	68.16	107.60	7.67	9.64	63.34	13.53	12.58
Fruit length (mm)	102.44	200.04	10.57	14.77	51.21	14.92	15.58
Peel thickness (mm)	3.79	7.09	19.32	26.43	53.42	2.93	29.09
Number of segments	0.27	1.05	3.98	7.69	26.72	0.55	4.23
Number of seeds	822.99	825.10	176.36	176.58	99.74	59.02	362.84
Vitamin C (mg/L)	6815.05	6848.50	18.03	18.08	99.51	169.64	37.07
$\beta$ -carotene (mg/L)	0.00	0.00	143.57	160.52	80.00	0.10	264.46
Juice yield (%)	40.92	40.97	18.55	18.56	99.88	13.17	38.20
Titratable acidity (%)	0.04	0.06	15.05	16.94	78.87	0.39	27.53
Total soluble solids (%)	0.77	0.81	10.14	10.39	95.34	1.77	20.41
Maturity index	0.42	0.44	10.51	10.69	96.68	1.31	21.29

Where GV = genotypic variance, PV = Phenotypic variance, GCV = Genetic coefficient of variation, PCV = Phenotypic coefficient of variation,  $H^2(B)$  = Broad-sense heritability, GA = Genetic advance, and GAM = Genetic advance percentage of mean.



In terms of fruit juice content, the grapefruit and pomelo genotypes were found to differ significantly ( $p<0.01$ ). The highest juice content (40.13%) was measured for the *Marsh* genotype, followed by the *Jouva* (39.36%) and *Triomphe* (38.57%) genotypes, while the lowest juice content (19.60%) was found for *Sidi Aissa* (Table 2). The highest (1.85%) and lowest (1.15%) acid content was recorded for the *McCarty* and *Triomphe* genotypes, respectively. The total soluble solids (TSS) content of the fruit also differed significantly between the grapefruit and pomelo genotypes. The highest TSS was recorded for the *McCarty* genotype (10.33% °Brix), followed by the *Marsh* genotype (9.47% °Brix), while the lowest TSS was recorded for the *Triomphe* (7.20% °Brix) and *Natsu Mikan* (7.80% °Brix) genotypes (Table 2). For vitamin C content, our analysis showed that the *Natsu Mikan* and the *Pubescent* genotypes provided the greatest average vitamin C content (591.20 mg/L and 542.85 mg/L, respectively), followed by *Sidi Aissa* with an average of 536.27 mg/L, compared with the *Marsh* and *Ruby* genotypes, which only had an average content of 361.90 mg/L and 381.64 mg/L respectively (Table 2). Similarly, the  $\beta$ -carotene content (mg/L) varied between the genotypes, with the highest being

0.181 mg/L in the *McCarty* grapefruit, followed by the *Marsh* genotype with 0.101 mg/L. The lowest  $\beta$ -carotene contents was found with the *Sidi Aissa* pomelos and the *Thompson* and *Triomphe* grapefruits ( $\leq 0.002$  mg/L), while the remainder of the fruits presented intermediate amounts of  $\beta$  -carotene (Table 2).

**Analysis of Variability Parameters:** The analysis of the variance among the 12 quantitative traits revealed that the mean squares were significant ( $p<0.001$ ) for all the studied traits (Table 3), indicating that there is a sufficient degree of genetic variability for almost all the studied traits, with the exception being the number of segments per fruit, so this notable genetic variability can be exploited through selection. The variation due to replication was not significant.

The extent of the variability (Table 4) among the genotypes was determined in terms of the phenotypic coefficient of variation (PCV) and genetic coefficient of variation (GCV). The PCV for all the characteristics was slightly higher than the GCV. The highest PCV was recorded for the number of seeds per fruit (176.58%), followed by  $\beta$ -carotene (160.52%) and fruit weight (24.60%). The GCV, meanwhile, was also found to be highest for the number of seeds per fruit

**Table 5. The phenotypic (rp) and genotypic (rg) correlation coefficients for the 12 physicochemical characteristics of the grapefruit and pomelo genotypes.**

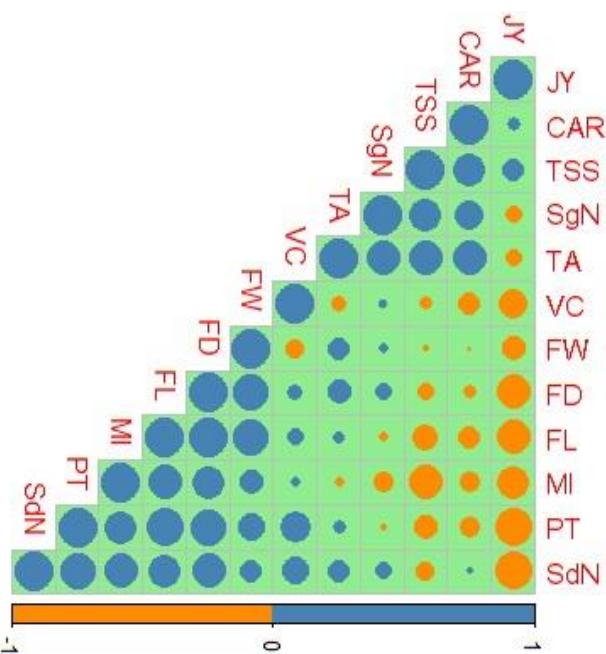
	FW	FD	FL	PT	SgN	SdN	VC	Car	JY	TA	TSS	MI	
FW	Rg	1	0.86**	0.82**	0.50 <sup>NS</sup>	0.20 <sup>NS</sup>	0.36 <sup>NS</sup>	-0.26 <sup>NS</sup>	0.002 <sup>NS</sup>	-0.38 <sup>NS</sup>	-0.06 <sup>NS</sup>	0.36 <sup>NS</sup>	0.43 <sup>NS</sup>
	Gp	1	0.8**	0.79**	0.47**	-0.01 <sup>NS</sup>	0.28 <sup>NS</sup>	-0.20 <sup>NS</sup>	-0.04 <sup>NS</sup>	-0.30 <sup>NS</sup>	0.01 <sup>NS</sup>	0.26 <sup>NS</sup>	0.31 <sup>NS</sup>
FD	Rg		1	1.08**	0.89**	0.23 <sup>NS</sup>	0.83**	0.17 <sup>NS</sup>	-0.08 <sup>NS</sup>	-0.84**	-0.22 <sup>NS</sup>	0.39 <sup>NS</sup>	0.71*
	Gp		1	0.80**	0.74**	0.14 <sup>NS</sup>	0.66**	0.12 <sup>NS</sup>	-0.14 <sup>NS</sup>	-0.67**	-0.09 <sup>NS</sup>	0.33 <sup>NS</sup>	0.53**
FL	Rg			1	0.99**	-0.01 <sup>NS</sup>	0.79**	0.22 <sup>NS</sup>	-0.33 <sup>NS</sup>	-0.86**	-0.53 <sup>NS</sup>	0.13 <sup>NS</sup>	0.86**
	Gp			1	0.73**	-0.11 <sup>NS</sup>	0.57**	0.16 <sup>NS</sup>	-0.30 <sup>NS</sup>	-0.61**	-0.26 <sup>NS</sup>	0.05 <sup>NS</sup>	0.58**
PT	Rg				1	-0.11 <sup>NS</sup>	0.98**	0.67*	-0.31 <sup>NS</sup>	-1.03**	-0.48 <sup>NS</sup>	0.14 <sup>NS</sup>	0.83**
	Gp				1	0.03 <sup>NS</sup>	0.72**	0.49**	-0.19 <sup>NS</sup>	-0.75**	-0.23 <sup>NS</sup>	0.08 <sup>NS</sup>	0.56**
SgN	Rg					1	0.25 <sup>NS</sup>	0.07 <sup>NS</sup>	0.70*	-0.27 <sup>NS</sup>	1.07**	1.02**	-0.37 <sup>NS</sup>
	Gp					1	0.14 <sup>NS</sup>	0.03 <sup>NS</sup>	0.39*	-0.15 <sup>NS</sup>	0.39*	0.54**	-0.22 <sup>NS</sup>
SdN	Rg						1	0.48 <sup>NS</sup>	0.03 <sup>NS</sup>	-0.92**	-0.25 <sup>NS</sup>	0.32 <sup>NS</sup>	0.72*
	Gp						1	0.48**	0.03 <sup>NS</sup>	-0.92**	-0.22 <sup>NS</sup>	0.31 <sup>NS</sup>	0.71**
VC	Rg							1	-0.34 <sup>NS</sup>	-0.55 <sup>NS</sup>	-0.12 <sup>NS</sup>	-0.15 <sup>NS</sup>	0.07 <sup>NS</sup>
	Gp							1	-0.31 <sup>NS</sup>	-0.55**	-0.12 <sup>NS</sup>	-0.15 <sup>NS</sup>	0.07 <sup>NS</sup>
Car	Rg								1	0.12 <sup>NS</sup>	0.71*	0.77**	-0.26 <sup>NS</sup>
	Gp								1	0.11 <sup>NS</sup>	0.60**	0.67**	-0.23 <sup>NS</sup>
JY	Rg									1	0.30 <sup>NS</sup>	-0.19 <sup>NS</sup>	-0.68*
	Gp									1	0.28 <sup>NS</sup>	-0.19 <sup>NS</sup>	-0.66**
TA	Rg										1	0.76**	-0.75*
	Gp										1	0.70**	-0.67**
TSS	Rg											1	-0.08 <sup>NS</sup>
	Gp											1	-0.07 <sup>NS</sup>
MI	Rg												1
	Gp												1

Where FW = Fruit weight, FD = Fruit diameter, FL = Fruit length, PT = Peel thickness, SgN = Segments Number, SdN = Seeds Number, VC = Vitamin C, Car = Beta-carotene, JY = Juice yield, TA = Titratable Acidity, TSS = Total soluble solids, MI = Maturity index. \*\* indicates significance at the 0.01 probability level. \* indicates high significance at the 0.05 probability level. NS indicates non-significance at the 0.05 probability level.



(176.36%), again followed by  $\beta$ -carotene (143.57%) and fruit weight (19.36%), indicating a greater degree of genetic variability between different genotypes for these traits. The greatest estimate of broad-sense heritability ( $H^2$ ) was found for Juice yield (99.88%), followed by Number of seeds (99.74%) and Vitamin C (99.51%). Genetic advance (GA) was found to be highest for vitamin C (169.64), followed by fruit weight (168.50) and the number of seeds per fruit (59.02), while the Genetic advance as a percentage of mean (GAM) was highest for the number of seeds per fruit (362.84), followed by  $\beta$ -carotene (264.46), juice yield (38.20), and vitamin C (37.07). The highest heritability (99.51) coupled with a higher GA (169.64) was found for vitamin C content.

**Analysis of the Coefficient of Correlation:** The correlation coefficients were calculated for the 12 genotypic and phenotypic traits (Table 5 and Figure 1). For most traits, the values of the genotypic correlation coefficients were greater than those of the phenotypic correlation coefficients, suggesting that there is a strong connection between distinct traits that is strongly influenced by the environment.



**Figure 1. Correlation coefficients of the traits for the grapefruit and pomelo genotypes.**

Where FW = Fruit weight, DF = Fruit diameter, FL = Fruit length, PT = Peel thickness, SgN = Number of segments, SdN = Number of seeds, VC = Vitamin C, Car = Beta-carotene, JY = Juice yield, TA = Titratable acidity, TSS = Total soluble solids, MI = Maturity index.

Mean fruit weight positively and highly significantly correlated with fruit diameter and fruit length at the genotypic and phenotypic levels, as well as with peel thickness at the phenotypic level only. Fruit diameter has a highly significant

positive correlation with fruit length, peel thickness, average number of seeds per fruit, and maturity index at the genotypic and phenotypic levels. Fruit length also positively and highly significantly correlated with peel thickness, average number of seeds per fruit, and maturity index at the genotypic and phenotypic levels. Peel thickness, meanwhile, positively and highly significantly correlates with the average number of seeds per fruit, vitamin C ( $rg=0.67^*$ ,  $rp=0.49^{**}$ ), and maturity index at the genotypic and phenotypic levels. The number of segments per fruit positively and highly significantly correlates with  $\beta$ -carotene ( $rg=0.70^*$ ,  $rp=0.39^{**}$ ), total soluble solids, and acidity at the genotypic and phenotypic levels. The average number of seeds per fruit positively and highly significantly correlates with the maturity index at the genotypic and phenotypic levels, as well as with vitamin C ( $rp=0.48^{**}$ ) at the phenotypic level alone. Juice yield highly significantly and negatively correlates with fruit diameter, fruit length, peel thickness, average number of seeds per fruit, and maturity index at the genotypic and phenotypic levels and vitamin C ( $rp=-0.55^{**}$ ) at the phenotypic level only. Total soluble solids was found to positively and significantly correlate with acidity ( $rg=0.76^{**}$ ,  $rp=0.70^{**}$ ) and  $\beta$ -carotene ( $rg=0.77^{**}$ ,  $rp=0.67^{**}$ ) at the genotypic and phenotypic levels. This stands out among the characteristics that are associated with  $\beta$ -carotene, because it indicates that fruit juices with greater total soluble solids tend to also have greater amounts of  $\beta$ -carotene. Acidity positively and highly significantly correlates with  $\beta$ -carotene ( $rg=0.71^*$ ,  $rp=0.60^{**}$ ) and total soluble solids ( $rg=0.76^{**}$ ,  $rp=0.70^{**}$ ) at both the genotypic and phenotypic levels. It also showed a negative and significant correlation with maturity index ( $rg=-0.75^{**}$ ,  $rp=-0.67^{**}$ ). The remaining relationships showed low correlation coefficients or were not significant.

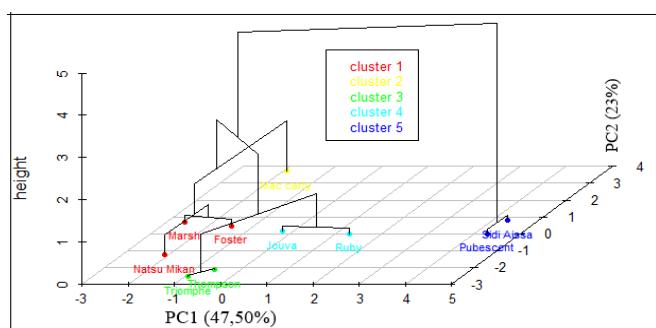
#### Multivariate statistical analysis

**Hierarchical Clustering of Principal Components:** The hierarchical clustering of the principal components allowed us to characterize two distinct profiles: The first profile includes four clusters among the grapefruit cultivars, namely 1) *Marsh, Foster, and Natsu Mikan*; 2) *McCarty*; 3) *Triomphe* and *Thompson*; 4) *Jouva* and *Ruby*. There was also a single cluster in the pomelo cultivars 5) *Sidi Aissa* and *Pubescent* (Figure 2). We found that the average weight in cluster 1 (i.e., *Marsh, Foster, and Natsu Mikan*) was significantly lower than it was for varieties in other clusters. *McCarty* of cluster 2 was characterized by the highest average  $\beta$ -carotenoid content and acidity, while *Thompson* and *Triomphe* of cluster 3 were similar in all the physicochemical characteristics. Cluster 4 had the highest average weight. In contrast, the seed number, peel thickness, maturity index, fruit length, and fruit diameter were significantly higher in cluster 5, although the juice yield was lower (Table 6).



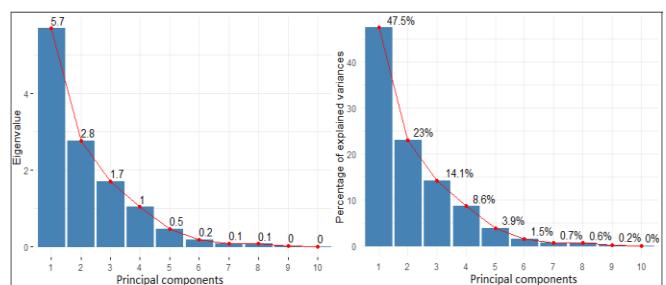
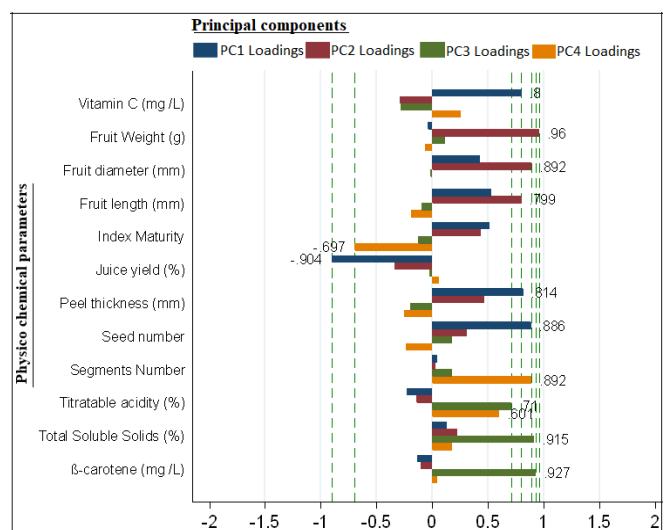
**Table 6. Clusters characterized by phenotypic traits from principal component analysis.**

Physicochemical Parameters	V. Test	Mean	Overall mean	SD	Overall SD	p-value
<u>Cluster 1 (representative varieties: Foster=0.76, Marsh=1.61 and Natsu Mikan=1.76)</u>						
Fruit weight (g)	-2.13	-1.02	-1.6e-17	0.43	0.95	<i>p</i> <0.05
<u>Cluster 2 (McCarty=0)</u>						
β-carotenoid (mg/L)	2.55	2.42	-4.85e-17	0	0.94	<i>p</i> <0.05
Titratable acidity (%)	2.00	1.90	-2.02e-16	0	0.94	<i>p</i> <0.05
<u>Cluster 3 (representative varieties: Thompson=0.31 and Triomphe 0.31)</u>						
Vitamine C (mg/L)			-			
<u>Cluster 4 (Ruby=0.80 and Jouva=0.80)</u>						
Fruit weight (g)	2.13	1.35	-1.66e-17	0.14	0.95	<i>p</i> <0.05
<u>Cluster 5 (representative varieties: Sidi Aissa=0.74 and Pubescent=0.74)</u>						
Seed number	2.95	1.86	2.50e-17	0.11	0.95	<i>p</i> <0.01
Peel thickness (mm)	2.77	1.75	0.000	0.001	0.948	<i>p</i> <0.01
Maturity index	2.33	1.47	-9.71e-18	0.21	0.943	<i>p</i> <0.05
Fruit length (mm)	2.24	1.42	-4.96e-16	0.035	0.94	<i>p</i> <0.05
Fruit diameter (mm)	2.044	1.29	-6.99e-16	0.22	0.94	<i>p</i> <0.05
Juice yield (%)	-2.78	-1.76	-4.94e-16	0.56	0.94	<i>p</i> <0.01

**Figure 2. Dendrogram for the hierarchical clustering.**

**Principal component analysis (PCA):** The PCA reduced the 12 physicochemical parameters (i.e., juice yield, seed number, peel thickness, vitamin C, fruit weight, fruit diameter, fruit length,  $\beta$ -carotene, total soluble solids, titratable acidity, segment number, and maturity index) into four orthogonal principal components (PC1, PC2, PC3, and PC4) with eigenvalues greater than one (1.027-5.701). These were extracted following the procedures associated with Kaiser's criterion. These components account for 93.182% of the total variance in the data matrix. PC1 explains 47.50% of the total variance with an eigenvalue of 5.70, with there being high positive loadings for seed number (0.886), peel thickness (0.814), and vitamin C (0.800) and a negative loading for juice yield (%) (-0.904). PC2 explained 23% of the total variance with an eigenvalue of 2.80, with there being positive loadings for fruit weight (0.960), fruit diameter (0.892), and fruit length (0.799). PC3 explains 14.10% of total variance with an eigenvalue of 1.70, with there being high positive loadings for  $\beta$ -carotene (0.927), total soluble solids (0.915), and titratable acidity (0.710). Finally, PC4 explain 8.60% of total variance, with an eigenvalue of 1.00, with there being a

high positive loading for number of segments (0.892) and a negative loading for maturity index (-0.697) (Fig. 3 and 4).

**Figure 3. Plot of the eigenvalues and the percentages of explained variance.****Figure 4. Varimax orthogonal rotation for the factor loadings from the PCA dataset of chemical and bioactive parameters.**

## DISCUSSION

The fruit weight among the pomelo and grapefruit genotypes varied from  $684.8 \pm 113.8$  g (*Jouva*) to  $362.6 \pm 73.5$  g (*Natsu Mikan*). A similar result had already been found for the *Oroblanco* cultivar (626.20 g) under dry tropical conditions in Mexico (Becerra-Rodríguez *et al.*, 2008). Indeed, the variation in fruit weight was more or less similar to that found by Nabi *et al.* (2004) in Pakistan, where eight grapefruit cultivars produced fruits weighing from 302.4 g to 506.5 g. This was less than those identified in Mexico, for *Gardner Marsh* (537.50 g) and *Reed Marsh* (556.20 g) by Becerra-Rodríguez *et al.* (2008). The results are further confirmed by those of (Roy *et al.*, 2014) in India, who reported a maximum fruit weight of 1350g for “pomelo type 4.” (Rahman *et al.*, 2003), meanwhile, reported that the fruit weight of pomelos varied from 718.33 g to 2160 g. In Bangladesh, the fruit weight varied from 396 g to 1418 g in one study (Hossain *et al.*, 2018). This variation may be due to genetic, physiological, and nutritional factors, as well as environmental influences (Hossain *et al.*, 2018).

Fruit diameter was significantly higher for *Sidi Aissa* ( $122.793 \pm 0.8$ ) and *Pubescent* ( $117.79 \pm 7.11$ ). Likewise, the fruit length was also significantly higher for *Sidi Aissa* ( $114.2 \pm 5.91$ ) and *Pubescent* ( $109.04 \pm 10.33$ ). Peel thickness was also the greatest for *Pubescent* ( $13.56 \pm 0.94$  mm) and *Sidi Aissa* ( $13.53 \pm 0.74$  mm), while it was the thinnest for *Marsh* ( $8.25 \pm 1.58$  mm) and *McCarty* ( $8.046 \pm 1.27$  mm). A similar result was found in an Indian study, which reported that the *Foster* (*Citrus paradisi*) cultivate had the greatest length and fruit diameter of 94.61 and 118.75 mm, respectively, while *Marsh seedless* had the lowest length and fruit diameter of 79.95 and 98.42 mm, respectively (Baswal *et al.*, 2015).

Regarding the nutritional attributes of the fruits, we found that the titratable acidity (%) varied considerably between the different cultivars. The lowest acidity ( $1.15 \pm 0.01$ %) was found for *Triomphe* and *Pubescent* ( $1.25 \pm 0.00$ ), while it was highest for *McCarty* ( $1.85 \pm 0.04$ %) and *Foster* ( $1.7 \pm 0.05$ %), followed by *Marsh* ( $1.56 \pm 0.01$ %) (Table 2). A similar result was found for French pomelo cultivars, with acidity ranging from ( $1.1 \pm 0.1$ %) to ( $2.2 \pm 0.3$ %) (Fanciullino *et al.*, 2006), while researchers studying Chinese genotypes reported acidities between 0.25 and 1.88% (Xu *et al.*, 2008).

The total soluble solids (°Brix) was lower for *Triomphe* ( $7.2 \pm 0.1$ ) than it was for *Marsh* ( $9.46 \pm 0.06$ ) and *McCarty* ( $10.33 \pm 0.30$ ). Very similar results were found in India, with them ranging from 7.3 to 8.7% (Ahmed *et al.*, 2018), while they varied between ( $7.29 \pm 0.03$ %) and ( $8.87 \pm 0.02$ %) in Pakistan (Usman and Fatima, 2018), between 8.25 and 8.81% in another study (Baswal *et al.*, 2016), and between ( $8.9 \pm 0.1$ ) and ( $10.8 \pm 0.2$ %) in a French study (Fanciullino *et al.*, 2006). Furthermore, total soluble solids has been reported as being between ( $11.60 \pm 0.01$  and  $12.27 \pm 0.06$ %) in a Spanish study (Zacarías-García *et al.*, 2021). Taking into account the

pomelo cultivars, a French study reported average total soluble solids (TSS) as being between ( $10.0 \pm 0.4$ %) and ( $11.5 \pm 0.5$ %) (Fanciullino *et al.*, 2006), while a Chinese study cited it as being between 10.33 and 11.92% (Xu *et al.*, 2008). These results illustrate how spontaneous mutations in a pomelo or grapefruit do not prevent it from maintaining the same genotypic character in terms of the total soluble solids in the fruit.

The analysis of vitamin C content showed that *Natsu mikan* and *Pubescent* provided the highest average vitamin C content of ( $591.2 \pm 9.00$  mg/L) and ( $542.85 \pm 0.4$ ), respectively, followed by *Foster* with an average of  $516.3 \pm 5.7$  mg/L. This compares with *Marsh* and *Ruby*, which only have an average content of 362 and 382 mg/L, respectively. In other work, it was found to be between 123.8 and 581.7 mg/L for eight grapefruit varieties in Pakistan (Nabi *et al.*, 2004), between 340 and 500 mg/L in an Israeli study (Gorinstein *et al.*, 2004), and between 330 and 430 mg/L in Turkey (Kelebek, 2010). Indeed, the grapefruit variety resulting from a spontaneous natural hybridization between pomelo and orange, followed by successive spontaneous mutations, had a positive effect on the vitamin C content of the *Natsu Mikan* variety, while the *Pubescent* variety’s deep mutation from a pomelo also had a positive effect on vitamin C content. In contrast, the *Sidi Aissa* variety retained almost the same genetic profile as pomelo cultivars. The  $\beta$ -carotene content ranged from 0.00 to 0.181 mg/L.

Two grapefruit varieties, namely *Jouva* (39.36 %) and *Marsh* (40.13 %), were found to have a significantly higher juice content when compared to the *Sidi Aissa* (19.60 %) and *Pubescent* (26.76 %) pomelo varieties. Very similar results were reported by (Ahmed *et al.*, 2018) and (Sharma *et al.*, 2015) in India and (Nabi *et al.*, 2004) in Pakistan, but our values are higher than those found in a French study (Fanciullino *et al.*, 2006). These results, which were reported by authors in various geographical locations, show that some grapefruit varieties still maintained a significant presence of certain nutritional qualities, which supports the notion that the dominant effect at play is genotypic rather than environmental (Rodrigo *et al.*, 2013). A significant variation in the number of seeds per fruit among the pomelo and grapefruit genotypes was observed in this present study. Finally, *Foster* (5.29) and *Natsu Mikan* (5.38) showed significantly lower maturity indexes than *Pubescent* (7.28). These results reflect those reported in a French study (Fanciullino *et al.*, 2006), but they are superior to the values observed in India (Ahmed *et al.*, 2018). In other work in India and China on pomelo cultivars, the maturity index was found to range from 10.13 to 20 (Nishad *et al.*, 2018) and from 14.44 to 17.09 (Xu *et al.*, 2008), respectively. This variation occurs because some genotypes are fast growing, so they mature earlier, while other slow-growing varieties tend to be late in maturing.

This phenotypic analysis of the data will be of great significance to citrus breeders, because it could help select



certain varieties as suitable parents for future breeding programs. For example, when desiring a variety with a low seed count and a high level of carotenoids, juice, and sugar together with a medium acid content, the *Marsh* grapefruit would be preferred. When aiming for a high vitamin C content, the *Natsu Mikan* grapefruit would be a preferred parent. When wanting varieties with the best quantitative characteristics—such as diameter, length, peel thickness, and high seed count—the *Sidi Aissa* and *Pubescent* varieties would be preferred. For varieties with a medium seed count and high levels of carotenoids, sugar, and acid content, combined with medium juice content, the *McCarty* grapefruit should be selected. The *Jouva* and *Ruby* pomelos had the highest weight of all the cultivars studied.

The significant value of the mean sum of the genotypic squares indicates that an environmental influence has caused considerable variation for the considered traits. We found the greatest variation for juice yield, number of seeds per fruits, vitamin C content, maturity index, total soluble solids, and  $\beta$ -carotene levels. All the characteristics among the studied genotypes can therefore be improved by selection. The most essential role of heritability in this genetics study of quantitative traits is its predictive role in determining the phenotypic value and serving as a guide to reproductive benefits (Falconer and Mackay, 1996). The GCV, combined with the heritability estimates, provides a robust guide to the amount of GA that can be expected due to phenotypic selection (Burton, 1952). The studied genotypes exhibited a wide variation for the considered traits, and we reported the greatest variation for juice yield, seed number, vitamin C, maturity index, total soluble solids, and  $\beta$ -carotene, thus supporting the hypothesis that it is possible to improve these traits further through selection.

Our analysis showed the genotypic coefficient of variance (CGV) to be particularly high for seed number and  $\beta$ -carotene, while lower values were observed for segment number and fruit diameter. The high GCV scores for seed number and  $\beta$ -carotene suggest that these are suitable traits for cultivating high-yield varieties through hybridization and the selection of subsequent generations. Recently, (Ahmed *et al.*, 2018) concluded that for grapefruits, the highest PCV and CGV values were found for number of seeds per fruit. Furthermore, Burton (1952) suggested that the GCV is associated with a high degree of heritability (80% or greater), which would indicate that selection could be effective in improving these traits. In contrast, for a characteristic with limited heritability (40% or less), selection may prove to be relatively difficult or practically unfeasible due to the environment having a masking effect on the genotypic effects. The estimates for the broad-sense heritability for all traits ranged from 26.72% (for segment number) to 99.88% (for seed number, vitamin C, and juice yield). Virtually the same results for heritability have been observed for seed number, vitamin C, and juice yield in other varieties of pumelo

(Ahmed *et al.*, 2019). In contrast in another study, fruit weight, fruit length, fruit diameter, segment number, and acidity have been found to be highly heritable unlike in our results, although our study agrees about TSS (Roy *et al.*, 2014). The GA estimate is very useful as a tool for selection when combined with estimates of heritability (Johnson *et al.*, 1955), because high GA values indicate additive gene action, whereas low values indicate non-additive gene action (Singh and Narayanan, 1993). Thus, heritability estimates will be more reliable when accompanied by a high GA (Panse and Sukhatme, 1957), because heritability is mainly due to additive genetic effects. The high genetic advance scores reflect the high degree of heritability estimates for seed number,  $\beta$ -carotene, juice yield, and vitamin C, indicating the existence of additive genetic effects, so selecting based on the phenotypic performance of these traits may be an effective method.

The genotypic correlation coefficients were higher than the phenotypic correlation coefficients, suggesting that there is a strong connection between distinct traits that are strongly influenced by the environment. This finding is consistent with the previous studies of (Nagariya *et al.*, 2015). The correlation coefficient revealed that peel thickness predicts vitamin C content, with it increasing along with peel thickness, although the juice yield decreases. The analysis also revealed that vitamin C increases with an increasing number of seeds per fruit. In addition, fruit juices with higher total soluble solids tend to have greater amounts of  $\beta$ -carotene.

In our examination of variance results within this study, we substantiated significant quantitative variability in the investigated traits. Simultaneously, the genotypic correlation analysis unveiled the environmental impact on the expression of characteristics across diverse genotypes. Moreover, the scrutiny of genetic variability suggested the feasibility of discerning crucial traits within the examined genotypes, including seed number,  $\beta$ -carotene, juice yield, and vitamin C levels. Conversely, hierarchical clustering analysis and principal component analysis facilitated the categorization of distinct class profiles characterized by similar quantitative attributes. Subsequently, we delineated these distinctive profiles among the studied genotypes; The *Pubescent* and *Sidi Aissa* group showcased characteristics such as high fruit weight, elevated vitamin C, moderately high average seed count, and slightly increased total soluble solids (TSS). However, it was notable for lower juice content and  $\beta$ -carotene levels. In contrast, *Foster*, *Marsh*, and *Natsu Mikan* were characterized by slightly reduced fruit weight, lower average seed count, elevated vitamin C (excluding *Marsh*), lower  $\beta$ -carotene (*Foster* and *Natsu Mikan*), and relatively higher juice content. Notably, the *Marsh* genotype recorded the highest vitamin C content and a relatively high total soluble solid. *McCarty* stood out with increased fruit weight, higher average seed count, low vitamin C, the highest  $\beta$ -carotene, slightly reduced juice content, and the highest total



soluble solids. *Thompson* and *Triomphe* exhibited moderately increased fruit weight, lower average seed count, low vitamin C, lower  $\beta$ -carotene, slightly elevated juice content, and slightly reduced TSS.

**Conclusion:** This study has revealed how the considered grapefruit and pomelo genotypes differ phenotypically, and this offers opportunities for genetic gain through selection or hybridization. For example, *Sidi Aissa*, *Pubescent*, and *McCarty* had the greatest seed counts, while *Jouva* and *Marsh* were juicier. Vitamin C, meanwhile, was most abundant in *Natsu Mikan* followed by *Pubescent* and *Sidi Aissa*. When considering  $\beta$ -carotene, juice content, and total soluble solids, we characterized *Marsh* and *McCarty* as the most acidic varieties. Our study concluded that the occurrence of additional gene action with little environmental impact is responsible for determining seed number,  $\beta$ -carotene, juice yield, and vitamin C relative to other studied traits. Furthermore, a strong correlation exists between vitamin C and the number of seeds per fruit, peel thickness and vitamin C, with another being found for fruit total soluble solids and  $\beta$ -carotene content, indicating that these traits could be used as criteria for genotype selection. This study reveals variations among grapefruit and pomelo genotypes, informing genetic improvement. Targeted breeding can decrease traits like seed count but increase vitamin C, beta-carotene, and juice yield. Correlations provide key indicators for genotype selection in breeding programs. Addressing real-world implications is crucial for practical application. Future research should focus on leveraging genetic insights for diverse citrus varieties.

**Authors' contributions:** Farid. E contributed to the design of the experiments, conducted literature reviews, and collected data. Farid. E, Mohamed. E, OUIAM. C, Abdelhak. T, Rachid. B, and Hamid. B carried out the experiments, performed statistical analyses, interpreted the results, and contributed to the writing and reviewing of the manuscript. All authors have read and approved the final version of the manuscript for publication.

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